Application No.: 10/537,075 Docket No.: 12810-00091-US

Amendment dated August 16, 2010 Reply to Office Action of April 27, 2010

## **AMENDMENTS TO THE CLAIMS**

## **Listing of Claims:**

1. (Currently amended) A method for expressing nucleic acid sequences in prokaryotic host cells by high-density cell fermentation comprising

- a) introducing into a prokaryotic host cell at least one DNA construct which is capable of episomal replication in said prokaryotic host cell and comprises a nucleic acid sequence to be expressed under the transcriptional control of an L-rhamnose-inducible promoter, wherein said L-rhamnose-inducible promoter is heterologous with regard to said nucleic acid sequence,
- b) selecting prokaryotic host cells which comprise said DNA construct in episomal form, and
- c) inducing the expression of said nucleic acid sequence by addition of L-rhamnose to a <u>high-density cell</u> culture of said selected prokaryotic host cells, wherein the concentration of L-rhamnose in the medium is from 0.01 g/l to 0.5 g/l,

wherein the prokaryotic host cell is at least deficient with regard to L-rhamnose isomerase.

- 2. (Original) The method according to claim 1, wherein the prokaryotic host cell is selected from the species of the family Enterobacteriaceae or the order Actinomycetales.
- 3. (Previously presented) The method according to claim 1, wherein the prokaryotic host cell is Escherichia coli.
- 4. (Previously presented) The method of claim 1, wherein the L-rhamnose-inducible promoter is the  $rhaP_{BAD}$  promoter from E. coli or a functional equivalent thereof or a functionally equivalent fragment of the promoter.
- 5. (Previously presented) The method of claim 1, wherein the L-rhamnose-inducible promoter comprises at least one RhaS binding element as shown in SEQ ID NO: 5 or a functional equivalent thereof or a functionally equivalent fragment of the above elements.

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6. (Previously presented) The method of claim 1, wherein the L-rhamnose-inducible promoter comprises at least one sequence described by SEQ ID NO: 1, 2, 3 or 4.

- 7. (Previously presented) The method according to claim 1, wherein the L-rhamnose isomerase is described by the amino acid sequence as shown in SEQ ID NO: 9 or a functional equivalent thereof.
- 8. (Previously presented) The method according to claim 1, wherein the DNA construct which is capable of episomal replication has a size of not more than 100 000 bases or base pairs.
- 9. (Previously presented) The method according to claim 1, wherein the DNA construct which is capable of episomal replication is selected from the group consisting of circular plasmid vectors, phagemids and cosmids.
- 10. (Previously presented) The method according to claim 1, wherein the prokaryotic host cell has at least one further deficiency with regard to a gene which has a function in the metabolization of rhamnose, where said gene encodes a protein selected from the group consisting of rhamnulose 1-phosphatase (RhaB) and rhamnulose-phosphate aldolase (RhaD).
- 11. (Previously presented) The method according to claim 1, wherein the expression of the nucleic acid sequence to be expressed causes the production of a protein encoded by said nucleic acid sequence.
- 12. (Previously presented) The method according to claim 1, wherein the nucleic acid sequence to be expressed encodes a recombinant protein selected from the group consisting of chymosines, proteases, polymerasen, saccharidases, dehydrogenases, nucleases, glucanases, glucose oxidases, α-amylases, oxidoreductases, peroxidases, laccases, xylanases, phytases, cellulases, collagenases, hemicellulases, lipases, lactases, pectinases, amyloglucosidases, glucoamylases, pullulanases, glucose isomerases, nitrilases, esterases, nitrile hydratases, amidases, oxygenases, oxymitrilases, lyases, lactonases, carboxylases, collagenases, cellulases, serum albumins, factor VII, factor VIII, factor IX, factor X, tissue plasminogen factors, protein C, von Willebrand factors, antithrombins, erythropoietins, colony-stimulating factors, cytokins, interleukins, insulins, integrins, addressins, selectins, antibodies, antibody fragments, structural proteins, collagen, fibroins, elastins, tubulins, actins, myosins, growth factors, cell-cycle proteins, vaccines, fibrinogens and thrombins.

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13. (Currently amended) A prokaryotic host cell which capable of producing recombinant proteins by high-density cell fermentation, wherein said host cell is at least deficient with regard to L-rhamnose isomerase and which-comprises at least one DNA construct, wherein the at least one DNA construct which is capable of replication in said host cell and which-comprises a nucleic acid sequence to be expressed under the transcriptional control of an L-rhamnose-inducible promoter in the presence of L-rhamnose at a concentration from 0.01 g/l to 0.5 g/l in high-dendity cell fermentation, wherein said L-rhamnose-inducible promoter is heterologous with regard to said nucleic acid sequence.

- 14. (Previously presented) A process for the production of foodstuffs, feedstuffs, enzymes, chemicals, pharmaceuticals or fine chemicals, which comprises utilizing the prokaryotic host cell of claim 13 for preparing foodstuffs, feedstuffs, enzymes, chemicals, pharmaceuticals or fine chemicals.
- 15. (Previously presented) A method for the production of recombinant proteins, enzymes and fine chemicals comprising using the prokaryotic host cell of claim 13 or a preparation thereof for producing recombinant proteins, enzymes and fine chemicals.